Captive breeding of the sea cucumber, *Holothuria scabra*, from India

Baskar D. James

Metha Nagar, Chennai, India

Abstract

This report deals with the hatchery and culture techniques of the sea cucumber, *Holothuria scabra,* from India. Larvae and juveniles were produced for the first time in 1988 at the Research Centre of Central Marine Fisheries Research Institute of Tuticorin on the south eastern coast of India. Large, healthy and uninjured specimens were selected as broodstock. They were stocked in one tonne tanks in the hatchery. Mud from the natural habitat was collected and put at the bottom of the tanks for the sea cucumbers to bury. The seawater in the tank was changed daily and the bottom mud was changed every fortnight. Sea cucumbers were subjected to thermal stimulation during March-May, the major breeding peak, and also during November-December, the minor breeding peak. The males released sperm within three hours of stimulation and were followed by females spawning about an hour later. The eggs were washed in fresh seawater and stocked at a density of 0.3 million eggs per 750 litres of seawater. The next day early auricularia larvae were developed. These larvae were fed on a microalgal culture of *Isochrysis galbana*. On the tenth day some of the auriculariae transformed into doliolariae. They were smaller than the auriculariae in size, highly motile and non feeding. After three days some of them transformed into pentactula larvae. They were fed on a mixed culture of *Chaetoceros calcitrans* and *Tetraselmis chuii*. The water in the tanks was changed daily, but the bottom was not cleaned to allow the algae to settle. After two months the juveniles reached a length of 20 mm.

These juveniles produced in the hatchery were grown in one tonne tanks, rectangular cages, velon screen pens and netlon screen pens, concrete rings at Karapad Bay, Valinokkam Bay and inside the harbour area for security. Best growth was noticed when the juveniles were grown in a prawn farm near Tuticorin. It is well known that much of the feed given to the prawns goes to waste, settling at the bottom of the farm enriching the farm soil, at the same time polluting the environment. The sea cucumbers are detritus feeders subsisting on the organic matter present in the substrate. The presence of the sea cucumbers at the bottom of the farm in no way affects the activities of prawn farming. In fact the prawns grow faster since the excess of food on the bottom is removed and the environment is kept cleaner by the presence of sea cucumbers. It is an ecofriendly practice which is beneficial both to prawns and sea cucumbers. In recent years the prawn farming industry in India has been rocked by disease and legal problems. The culture of sea cucumbers in prawn farms comes as a bonus for the prawn farmers.

Keywords: Broodstock, larval rearing, juvenile rearing, prawn farming

印度糙海参（*Holothuria scabra*）的人工育苗和养殖技术的研究

D.B.詹姆士

印度Chennai市的Metha Nagar县

摘要

本文介绍印度产的糙海参（*Holothuria scabra*）的育苗和养殖技术。第一次的育苗成功是1988年，由印度东南沿海的土第考林水产研究所完成的。个体大而健康，未受伤的个体被选作亲本。亲本被暂养在育苗场1吨的水池里。将亲本采集地的海泥铺在池底供海参潜伏。每天更换海水，海泥每两周更换一次。3-5月间采用温度诱导容易奏效，是产卵的高峰季节，而11-12月份是产卵的低谷。在温度诱导后的3小时内，雄性先排精，大约1小时后雌性也开始产卵。 受精卵用新鲜的海水洗后，在750升的水池内放30万粒卵。第二天早晨，受精卵发育到早期耳状幼虫。幼虫的饵料系等鞭金藻。第十天幼虫变态为樽形幼虫。在体形上樽形幼虫小于耳状幼虫。它们活跃地游动，但不摄食。3天后，部分樽形幼虫变态为五触手幼体。 此阶段幼体

的饵料是角毛藻和扁藻。此时仍然每天交换海水，但是池底无须每天清洗，因为有藻类饵料沉积在池底。两个月后，幼参体长达到20毫米。在育苗池内培育的幼参被分别放养在位于卡拉帕（Karapad）湾、法里努卡（Valinokkam）湾和港口内的1吨的水池、长方形网箱、维纶网围、塑料网围和混凝土圈内。最好的生长记录是养在靠近土第考林的虾池内的幼海参。众所周知，虾池内的残剩饲料在虾池内造成富营养化，污染水体。海参是以有机碎屑作为食物的。虾池内所养的海参不会对对虾产生不利影响，相反，海参为虾池清除掉过多的残饵，有助于对虾的生长。因此，海参与对虾养在同一池塘内形成生态互利的关系。近年来，对虾养殖业由于受到疾病的危害和法律的限制一直是踌躇不前。在虾塘内养殖海参为对虾养殖带来了实惠。

关键词：亲本、幼虫培养、幼参培养、对虾养殖

Introduction

According to the latest report of the Food and Agriculture Organisation (FAO) on world capture fisheries, production of sea cucumbers during 2001 was 18 900 tonnes (Vannuccini, 2004). India exported 50 tonnes of processed sea cucumber in 1989, valued at US$ 0.2 million. At present, the Government of India has completely banned collection, processing and export of all species of sea cucumbers from India as a conservation measure. According to Hornell (1917) the beche-de-mer industry in India is an ancient one, having been introduced by the Chinese more than one thousand years ago. Since sea cucumbers do not offer resistance at the time of capture and also do not make any attempts to escape, the resource is over exploited in many parts of the world. A classic example of this case is the beche-de-mer industry in the Maldives, which developed in 1985 with a modest export of 31 kg. Within 5 years the resource was in danger of over exploitation (Joseph and Shakeel, 1991; Joseph, 1992). James and James (1994a,b) wrote on the needs for conservation and management of sea cucumbers in India. A new resource of sea cucumber, *Actinopyga echinites*, was exported for the first time from India in 1989 and by 1992 this resource had become scarce (James and Badrudeen, 1995). Another species, *A. miliaris*, was collected for the first time in 1992 and, within 2 months, more than 0.6 million specimens were caught. James and James (1994) published a handbook on Indian sea cucumbers to facilitate identification of commercially important sea cucumbers from India. James and Ruparani (1999) gave an account of new resources of beche-de-mer and the management of these resources in India. In order to replenish the natural populations, hatchery technology for juvenile production and sea ranching was developed by the Central Marine Fisheries Research Institute in India.

The Chinese and Japanese are pioneers in the aquaculture of the sea cucumber, *Apostichopus japonicus*. Apart from these two countries some work has been completed on the production of sea cucumbers by the Koreans and the Russians. James *et al.* (1988) produced juveniles of *H. scabra* for the first time in 1988 at Tuticorin in India. Following this same technology, juveniles of this species have been produced in Australia, Indonesia, New Caledonia, Maldives, Solomon Islands and Viet Nam in recent years.

Hatchery production

Apostichopus japonicus juveniles were produced more than 60 years ago in Japan (Inaba, 1937) and were successfully reared (Imai *et al.*, 1950). Subsequently, Shuxu and Gongchao (1981), Li (1983) and Shui *et al.* (1986) reported on the breeding and culture of this species in China. In recent years there have been several authors from China and Japan who have worked on the artificial breeding of this species. James *et al.* (1988) produced *Holothuria scabra* juveniles for the first time in India. Since 1988 this species has been bred in captivity on a number of occasions (James 1993a, 1997, 1998; James and James, 1993; James *et al.* 1994a, b). Chen and Chan (1990) reported on the larval development of *Actinopyga echinites* and James *et al.*, (1993) reported on the spawning of *A. mauritiana*, while Asha and Rodrigo (2001) and Asha and Muthiah (2002) reported on spawning and larval rearing of *Holothuria spinifera* in India.

Collection of broodstock

Large and healthy broodstock specimens (Figure 1) were collected from the commercial catch destined for processing. Most individuals were collected by divers in shallow waters up to a depth of 10 m. The diving season for sea cucumbers is from October to March in the Gulf of Mannar and from March to October in Palk Bay.

Maintenance of broodstock

After collection, individuals were brought to the hatchery and stocked in one tonne tanks (Figure 1). Usually 15-20 specimens were stocked in an area of 2 m^2. The bottom of the tank was covered with mud from the natural habitat to a thickness of 15 cm to allow the broodstock to bury themselves. The water in the holding tanks was changed each morning and the mud at the bottom of the tanks changed every fortnight. Before use, the tanks were thoroughly cleaned with bleaching powder and put out in the sun to dry.

Figure 1. *Broodstock of Holothuria scabra.*

Thermal stimulation

Best results for thermal stimulation were obtained only during the breeding peaks (March-May) of the sea cucumbers. The temperature of the water was raised by 3-5 ˚C by slowly adding hot seawater and stirring uniformly. Usually 20 specimens were introduced into the tanks at 10:00 hrs. By 13:00 hrs the males had released sperm by raising the anterior end (Figure 2). One hour or so after the males had released sperm, the females started releasing the oocytes. Simultaneously, several males in the tank were still releasing sperm. It is better to keep only one male spawning in the tank, otherwise the over-abundance of spermatozoa clouds he water.

Figure 2. *A mature* Holothuria scabra *male in the act of spawning.*

Spawning behaviour

In sea cucumbers the sexes are separate but it is not possible to separate the males and females from an external examination in most species. In the case of *H. scabra*, only a microscopic examination of the gonads reveals whether the specimen is a male or a female. However, it is possible to distinguish them at the time of spawning since the spawning behaviour of the males and females is different. Typically, males spawn first, followed by females. The male first lifts the anterior end (Figure 2) and exhibits swaying movements just like a snake. After exhibiting such movements for some time the males start releasing the sperm in a fine white stream from the gonopore situated at the anterior end and in the mid-dorsal position. When a male starts releasing the sperms it continues for nearly 2 hours. Meanwhile the ripe females start reacting, possibly to the presence of sperm released in the water. The anterior ends of females become bulged due to the pressure created inside the gonopore by the accumulation of oocytes. The female releases the oocytes which settle down on the bottom of the tank. Sometimes the same females spawn for a second or even third time; this is mainly observed in large specimens. The oocytes are ejected out through the single gonopore. They are ejected out in a powerful jet reaching a distance of about one metre, helping in the dispersal over a wide area. The gametes are released as a light yellow mucus-like substance.

Fertilization

The fertilization is external and takes place in the water column. The oocytes are fertilized quickly as they make contact with the spermatozoa. After the oocytes and sperm are released in the water, the sea cucumbers are removed from the tank. The eggs are washed in fresh seawater several times to remove the excess spermatozoa. Excess spermatozoa can reduce the rate of fertilization and cause the development of deformed embryos.

Early development

Large females can release about one million oocytes. About 0.7 million eggs were stocked in 750 litres of water. The eggs were spherical, white and visible to the naked eye and were found floating in the water. The diameter of the oocytes ranged from 180 to 200 µm. After fertilization the eggs underwent cleavage and developed into the dipleurula stage with eggs ranging in length from 190 to 250 µm. The dipleurula transformed into early auricularia larva after 24 hours. They are 430 µm long and 280 µm wide at this stage.

The early auricularia larvae have a buccal cavity, ciliary bands, a cloaca and an anus and they actively feed. They were fed on the microalgae, *Isochrysis galbana,* and a mixed culture dominated by species of *Chaetoceros* spp. and *Skeletonema* spp. As days passed the auricularia (Figure 3) became more and more transparent and the lateral projections also became prominent. On each side of the late auricularia larva, four lateral projections were seen and at the end of each projection there was a hyaline sphere. The oesophagus and the pear-shaped stomach were well demarcated. Right and left stomatocoel were clearly seen. The ciliary bands showed a number of pigment spots. The length of the late auricularia larvae varied from 660 to 1050 µm (with an average of 860 µm) and the width was 240-690 µm (with an average of 500 µm). Some of the auricularia larvae remained small.

A few of the late auriculariae transformed into doliolariae (Figure 4) on the tenth day. The doliolariae were barrel-shaped with five hyaline spheres on each side. Later, the first two tentacles developed at the anterior end. Their length varied from 420 to 570 µm (with an average of 485 µm) and 240-390 µm in breadth (average of 295 µm).

On the thirteenth day, some of the doliolariae transformed into pentactula larvae (Figure 5). The body of the pentactula was tubular with five tentacles at the anterior end and with one short stumpy tube foot at the posterior end which helps in locomotion. The anal opening was distinct. The length varied from 330 to 750 µm (average of 307 µm). By the eighteenth day the tube feet and tentacles became distinct. Two long tube feet developed at the posterior end. At this stage the length of the juveniles was 550-720 µm (average of 656 µm) and the width varied from 210 to 320 µm (average of 262 µm). The pentactulae had the habit of moving to the edge of the tank, remaining just below the surface of the water. They soon settled down on the bottom of the tank.

Figure 3. Sea cucumber auricularia stage.

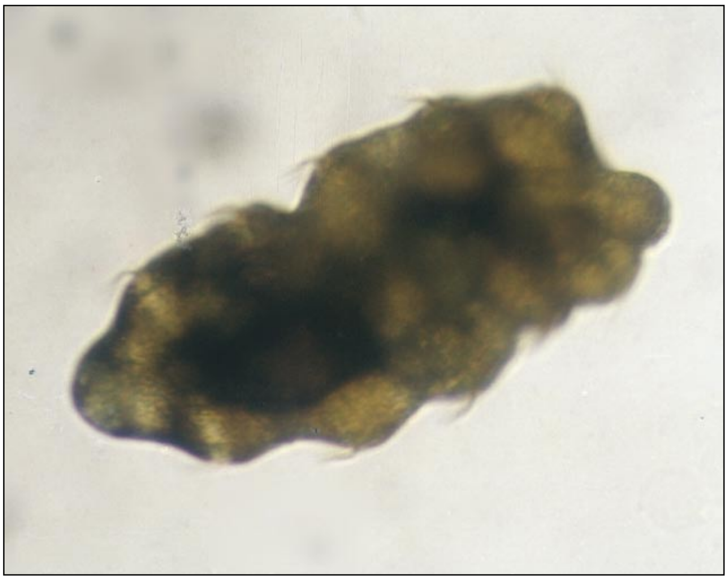

Figure 4. Sea cucumber doliolaria stage.

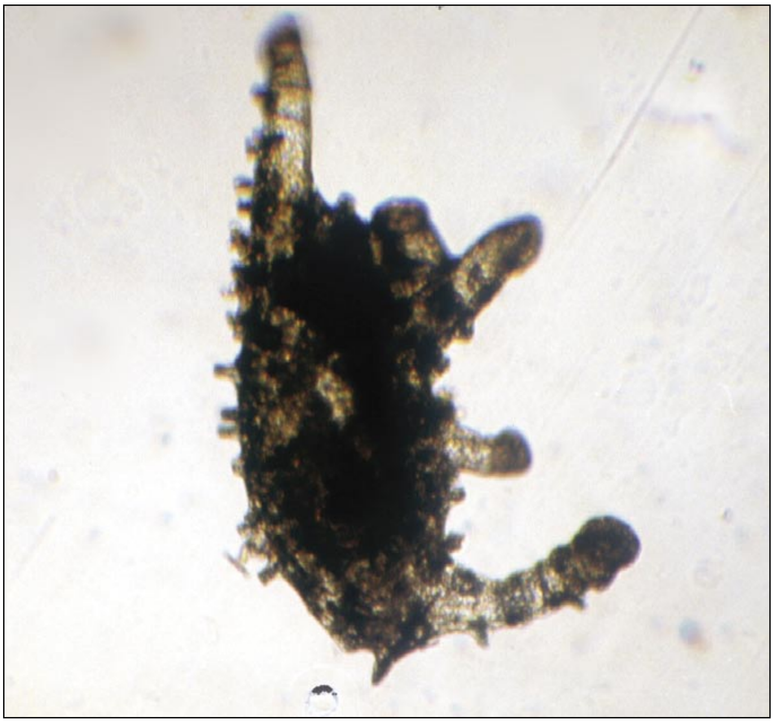

Figure 5. Sea cucumber early juvenile stage.

Rearing of the post larvae

Preparation of the rearing tanks

All rearing tanks used in breeding, especially new tanks must be scrubbed clean and filled with seawater for 20 days. During this period the water in the tanks is repeatedly changed. Before the tanks are used, they are then scrubbed and filled with seawater containing 40 ppm bleaching powder and then washed clean with filtered seawater.

Rearing density

Strict control over the rearing density of the larvae (i.e. the number of larvae per ml of water) is maintained. At present there are two methods used to rear the larvae: still water rearing and flowing water rearing. Auricularia larvae during their early and middle stages concentrate at the surface of the water. If the density of the larvae is high, they will form agglomerations and sink to the bottom of the tank resulting in their death. Rearing density, therefore, should be controlled to ensure better survival rates. The desirable density of auricularia is 300-700 per litre. In a one tonne tank filled with 750 litres of water, 0.3 million auriculariae can be reared.

Selecting and counting of larvae

After the embryos are transferred to rearing tanks, they develop into auricularia larvae in about 30 hours. Healthy larvae occupy the surface layer of the water while deformed larvae and dead embryos are found in the lower layer of the water column or on the bottom of the tank. A sample can be removed for counting the larvae. Samples can be taken separately from the two ends and the middle of the tank using 250 ml beakers. The sample is stirred and a 1 ml aliquot is taken with a pipette and placed on a plankton counting chamber to assess the number of larvae. Two more samples are taken and the average of three counts is taken as an indication of the density of the larvae. When the auricularia larvae are in the early stage of development, they should be reared at a density of about 500 larvae per litre. The development of the auricularia can be divided into three stages: early, middle and late.

Water management

During their development, the larvae eject faeces and consume dissolved oxygen constantly. Some of the larvae will die. These, together with excess food, will produce harmful substances such as hydrogen sulphide and ammonia. In addition, bacteria reproduce rapidly with the rise of temperature. Poor water quality directly affects the normal development of the larvae. Therefore proper water management and sanitation is essential, including regular cleaning of the tanks and frequent changing of water. Sediment and deformed larvae at the bottom of the tank have to be siphoned out preferably on a daily basis.

During water changes a sieve (80 µm mesh size) is used to prevent loss of eggs and/or larvae. While the water is being changed, it is advisable to constantly stir the water lightly around the tank. This will prevent damage to the larvae during the water change as without stirring the larvae would be forced into the sieve causing mechanical injury.

Larval feeding and feeding rates

High quality microalgae and proper feeding schedules are key factors in the successful rearing of sea cucumber larvae. Early auriculariae possess a well formed alimentary tract and must be fed. Ingestion by these larvae consists of conveying the suspended particles of food into the alimentary canal through the mouth parts by the swaying of peristomial cilia. The effectiveness of *Isochrysis galbana*, *Dunaliella salina, Dicrateria* spp. and mixed diets consisting of all the above mentioned microalgae was tested. The best growth rates and lower mortality were observed when larvae were first fed *I. galbana* and supplemented with mixed cultures, chiefly consisting of *Chaetoceros* spp., four or five days later. Unicellular algae were given twice a day but the amount given depended on the particular stage of the larvae. In general, a concentration of 20 000 to 30 000 cells per ml was maintained in the rearing tank. The amount of food given should be increased or decreased depending on the abundance of food observed in the stomach of the larvae. This can be visually assessed everyday before feeding.

Environmental factors

Monitoring of the environmental factors is important since the larvae and the juveniles are sensitive to environmental changes.

Temperature

At Tuticorin, the temperature of the seawater ranged from 26 to 30 ˚C. The optimum temperature for rearing of the larvae was found to be 27-29 ˚C. The temperature of the water should be recorded twice a day - once in the morning and once in the afternoon.

Dissolved oxygen

Dissolved oxygen (DO) levels vary with water temperature. The higher the temperature the lower the DO level. At Tuticorin, the normal DO level was around 5-6 ml per litre. Constant aeration was provided to the larval tanks throughout the day to make sure the oxygen level did not decrease. For a 1 tonne tank, two aerators are generally provided, one at either end.

pH

Under normal conditions the seawater is alkaline with a pH of 7.5-8.5. Tests have shown that the larvae adapt to a fairly wide range of pH. However when the pH rises above 9.0 or drops below 6.0, the movements of the larvae weaken and the growth stops. Therefore the pH of the water must be maintained between 6.0 and 9.0.

Salinity

Salinity of normal seawater at Tuticorin ranges from 31 to 34. The lethal critical salinity is 12.9, whereas the optimum salinity for larval development ranges from 26.2 to 42.7. In this range the higher the salinity the quicker the development. Extreme salinity levels adversely affect the normal development of embryos and larvae, resulting in a large number of deformed larvae and death.

Ammoniacal nitrogen

The ammoniacal nitrogen content of seawater is very low. The main sources in breeding tanks are the metabolites of the larvae, excess food and decomposing organisms. Accumulation of ammonia in concentrations above 500 mg/m^3 can be harmful for the larvae. The larvae can develop normally with ammoniacal nitrogen in the range of 70 to 430 mg/m^3 of water.

Rearing of juveniles

The pentactula settles when food is sufficient and also when a hard substratum is available. If these two conditions are not satisfied the pentactula continue to swim in the tank for long periods.

Types of settling bases

Two types of settling bases have been tried. Rough surface tiles were used in the first case. Filtered seawater was circulated in the tanks continuously for four or five days in good sunlight. Tiles were suspended in the water. After settlement of benthic algae on the tiles, the tiles were taken inside the hatchery and suspended in the tanks holding the doliolaria larvae. The hard surface and available food induced the doliolaria to metamorphose into pentactulae and subsequently settle on the tiles. One disadvantage with tiles of this type is that the benthic algae can die and easily come off after four or five days particularly in shady conditions.

An alternative settling method is to use polythene sheets that are kept in the tank filled with seawater. An algal extract, usually *Sargassum* spp. that has been filtered through a 40 µm sieve, is added to the tank. The algal extract sticks to the polythene sheets. The seawater is changed daily and fresh algal extract added in small quantities. After four or five days the polythene sheets become covered with a fine coat of algal extract and this serves as a good settling base for the larvae.

Juvenile diet

Newly metamorphosed juveniles have limited swimming ability and their tentacles are short. Seaweeds like *Sargassum* spp. and *Halimeda* spp. were tried as food. Protein rich *Sargassum* spp. and the sea grass, *Syngodium isoetifolium,* have been found to be suitable feed for the juvenile sea cucumbers. The algae are cut into small pieces and then grounded into a fine paste which is then filtered using a 40 µm sieve. After one month of growth, the paste is filtered through an 80 µm sieve. This filtered extract is given to the juveniles twice a day, in the morning and evening. The juveniles were found to feed actively on the settled algal extract and grew well.

Density of settled juveniles

When the larvae develop into juveniles they begin to crawl on the substrate with most of them remaining on the settlement plates. Fifteen days after settlement they can be clearly seen with the naked eye. At this stage the number of juveniles should be estimated. The sampling area of each tank must account for over 5 % of the total surface area. In order to achieve increased survival rate, it is necessary to maintain the settling density at the optimum level between 200 and 500 juveniles/m^2. Higher densities and insufficient food will adversely affect growth and survival. Juveniles reach 20 mm in length after two months (Figure 6) and 40 mm after four months (Figure 7).

***Figure 6.** Two-month old hatchery produced sea cucumber juveniles.*

Figure 7. *Four-month old hatchery produced sea cucumber juveniles.*

Predators and their control

Predation

Harpacticoids and other copepods and ciliates are the main predators of slow moving auricularia larvae. The predators usually attack the auricularia causing injuries and eventually killing them. They also harm the juveniles by reproducing rapidly in the rearing tanks and thus competing for food. Algal extracts of *Sargassum* spp. given for the juveniles are often found in the alimentary track of the copepods.

Predator control

The effectiveness of various chemicals was tested for the control of Harpacticoids and other copepods. Harpacticoids are sensitive to organic phosphorous and thus Dipterix, Kogor and other chemicals containing organo-phosphorous are effective. It was found that Harpacticoids can be killed with 2 ppm Dipterix in two hours with no harmful effects on the juveniles. The Dipterix solution should be evenly sprinkled into the tank and the water changed completely after two hours otherwise the chemicals may affect the juveniles.

Sea ranching

As stated in the introduction, a sea cucumber resource can quickly become over exploited since the animals do not make any attempts to avoid collection and do not have any effective defensive mechanism. This has made the sea cucumbers very vulnerable and natural populations have dwindled alarmingly as a result of overfishing. The juveniles produced in the hatchery are used for sea ranching in natural beds of sea cucumbers so that the natural population can be replenished. Trials are currently being conducted to test the effects of sea ranching in a particular area. James (1993b) provides details of sea ranching of sea cucumbers.

Future prospects

Studies conducted in India showed that the sea cucumber juveniles and young adults of *Holothuria scabra* grow relatively fast in prawn farms by making use of the feed waste. The growth of the sea cucumber juveniles is three times faster when they are grown in the prawn farm without affecting the normal prawn farming activities. If juvenile *H. scabra* are produced in good numbers it is advisable to release them directly into the farm at the rate of 30 000 juveniles/hectare. The growth rate is expected to be better when freely grown in the prawn farm rather than in a confined space like a concrete ring. The juveniles are expected to reach harvestable weight at the end of one year.

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